# Correlation analysis of paired leaf transcriptome and microbiome across diverse maize lines

Renato Augusto Correa dos **Santos**[1,2,3], Diego Mauricio **Riaño-Pachón**[1,2], Jason G. **Wallace**[3]

1 Laboratory of Computational, Evolutionary and Systems Biology, Center of Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil
2 National Institute for Science and Technology of Bioethanol, São Paulo, Brazil
3 Wallace Lab, Department of Crop & Soil Sciences, University of Georgia, Athens, GA, United States of America
* correspondence: renatoacsantos@gmail.com
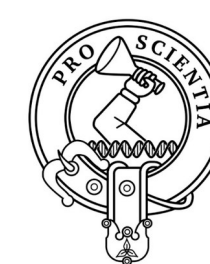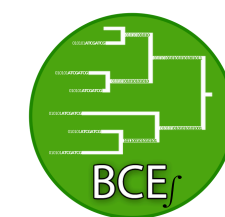
*Presenter*
Dr. Renato A. C. dos **Santos**

## Background

- Maize is an important crop worldwide

- Associations between transcriptome and microbiome can guide crop improvement

- User-friendly web platoforms enables exploitation of association by researchers

## Results

- Few ASVs recovered, probably due to low quality

- High quality mapping of trancriptome data

- Correlations between OTU counts and transcripts (TPM)

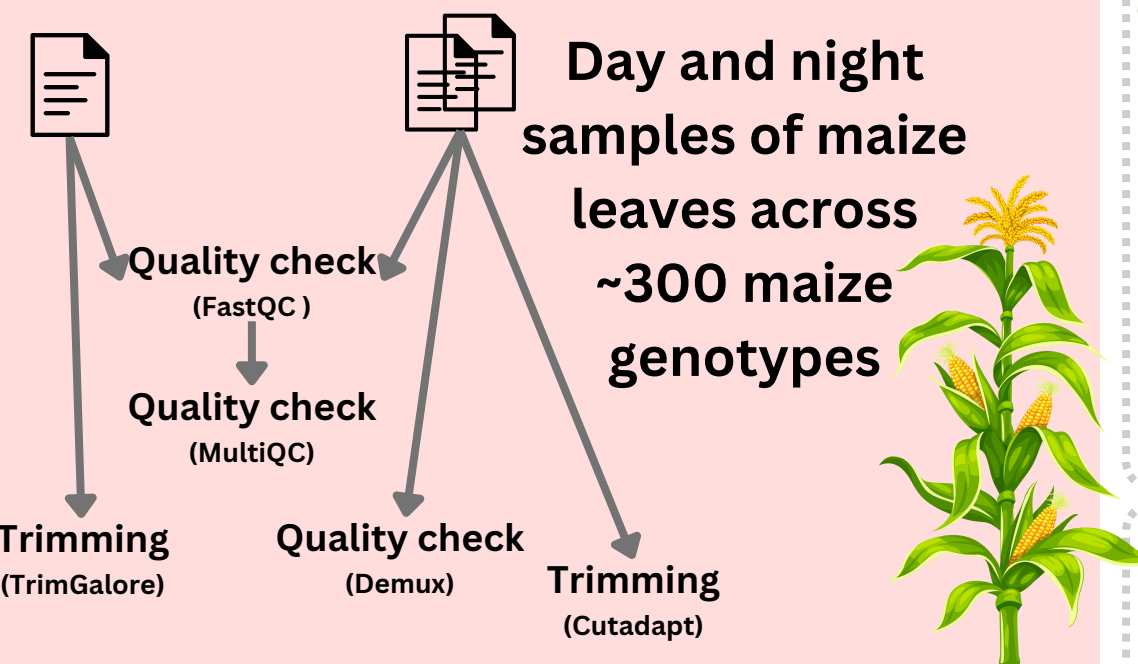- Initial implementation of CoNekT Grasses Microbiome

## Future directions

- Improve data transformation and use different strategies to investigate associations
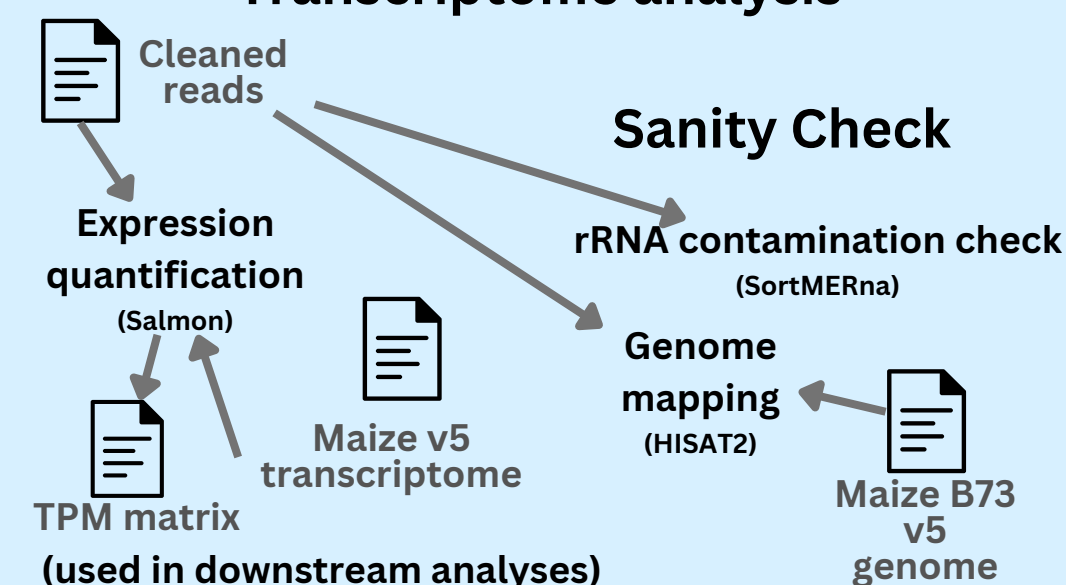
- Novel functions in CoNekT Grasses Microbiome

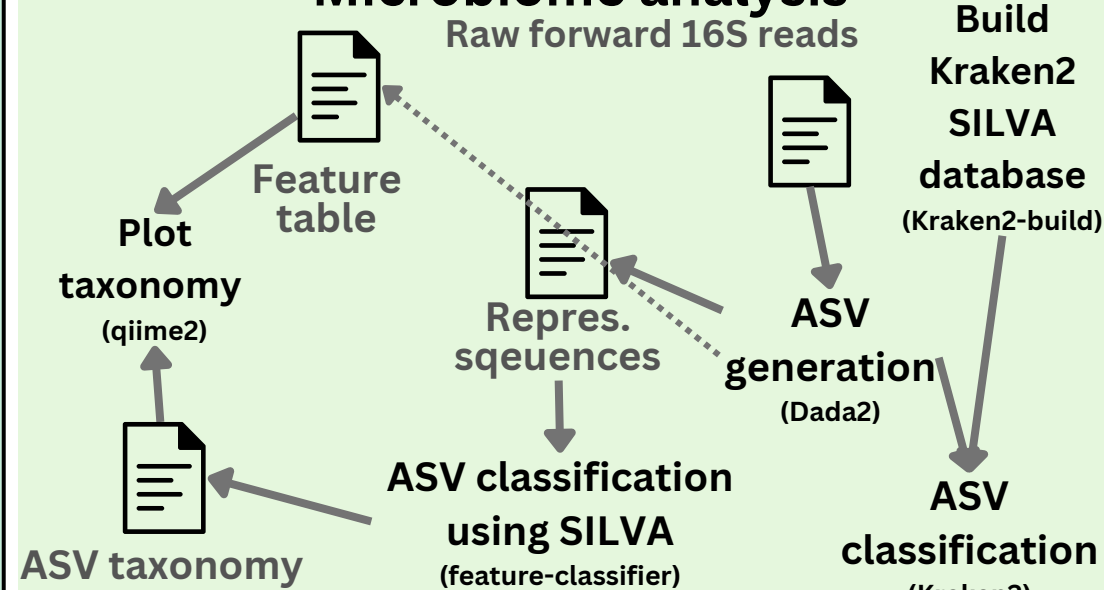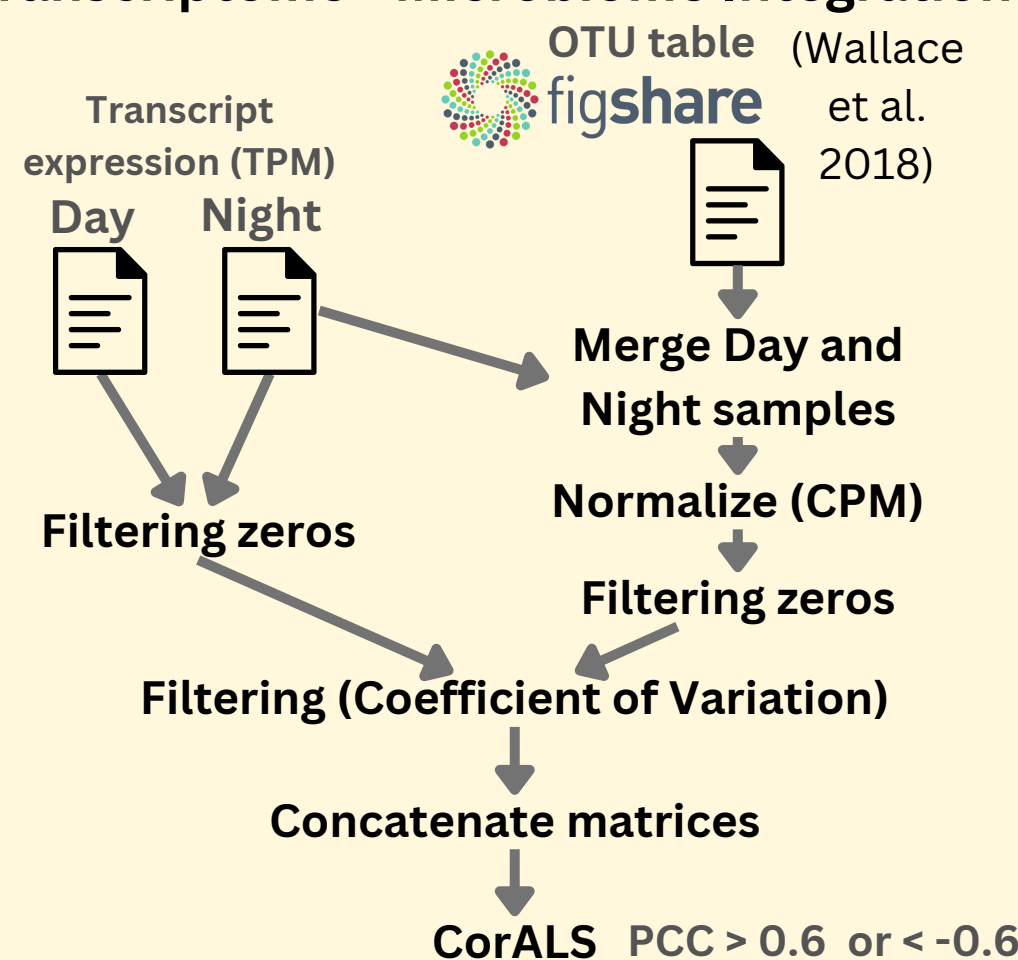## Main workflow

### Quality Check and Trimming

**RNAseq**  **Microbiome**

**Day and night samples of maize leaves across ~300 maize genotypes**

Quality check (FastQC)
Quality check (MultiQC)
Trimming (TrimGalore)
Quality check (Demux)
Trimming (Cutadapt)

### Transcriptome analysis

Cleaned reads

**Sanity Check**

Expression quantification (Salmon)
rRNA contamination check (SortMERna)
Genome mapping (HISAT2)
Maize v5 transcriptome
Maize B73 v5 genome
TPM matrix **(used in downstream analyses)**

### Microbiome analysis

Raw forward 16S reads
**Build Kraken2 SILVA database (Kraken2-build)**
Feature table
Repres. sqeuences
Plot taxonomy (qiime2)
ASV generation (Dada2)
ASV taxonomy
ASV classification using SILVA (feature-classifier)
ASV classification (Kraken2)

### Transcriptome - Microbiome Integration

Transcript expression (TPM)
**Day**   **Night**
OTU table **figshare** (Wallace et al. 2018)

Filtering zeros
**Merge Day and Night samples**
**Normalize (CPM)**
**Filtering zeros**
**Filtering (Coefficient of Variation)**
**Concatenate matrices**
**CorALS** PCC > 0.6 or < -0.6

## Results

### Attempt to analyze the microbiome with ASVs



Good / Poor
Mostly unassigned
Sequence base positions    Samples
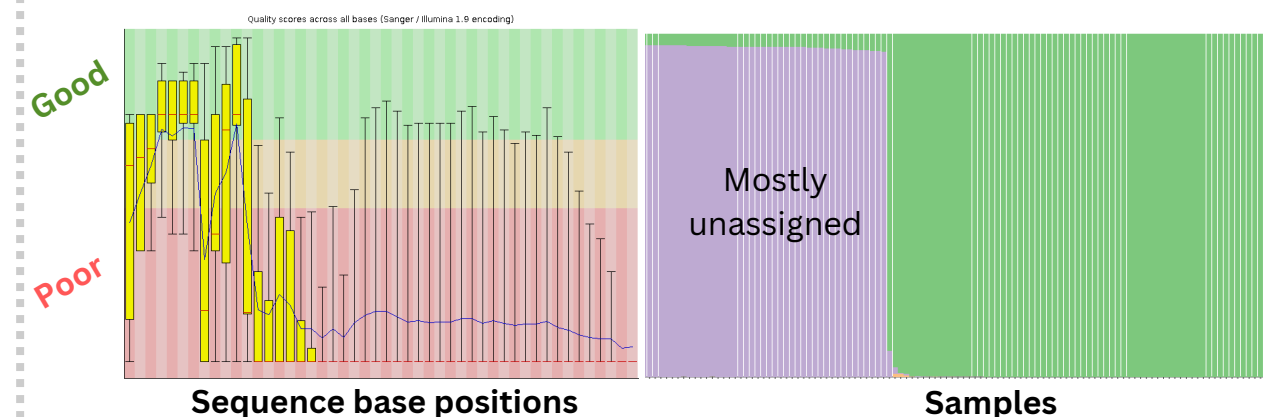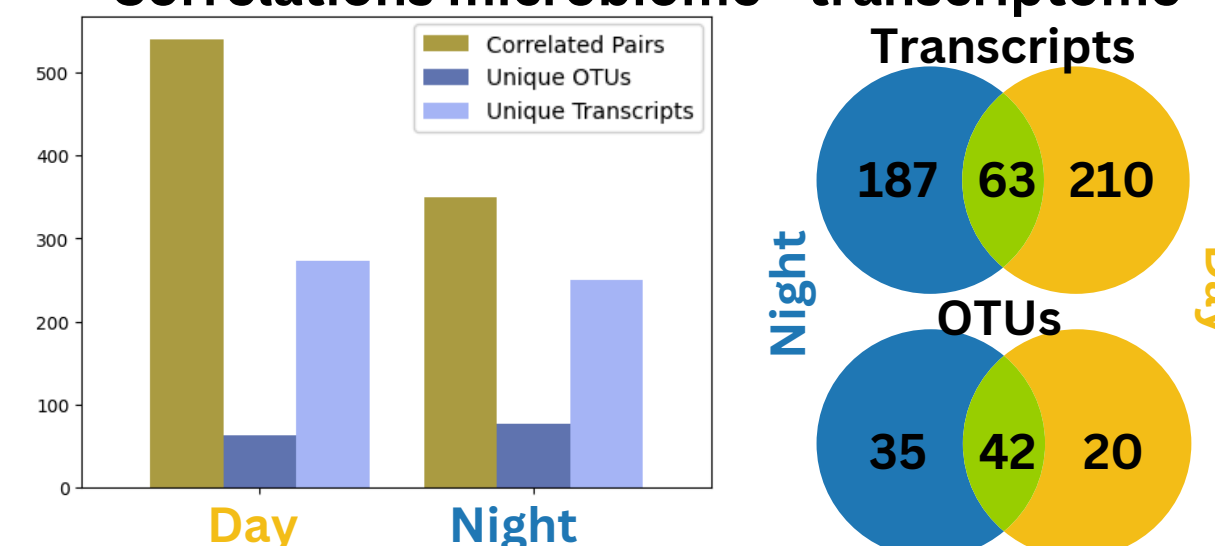
Low classification rates (right) probably associated with low read quality (left).

### RNA-Seq (host transcriptomics) and OTU table



OTU Table (16S)
RNAseq
*Pearson* correlation coefficients

HISAT2 (Maize v5), SortMERna, Kraken2 (maize), Kraken2 (unclassified), Salmon (Maize v5 primary transcripts), Salmon (Maize v5 all transcripts)
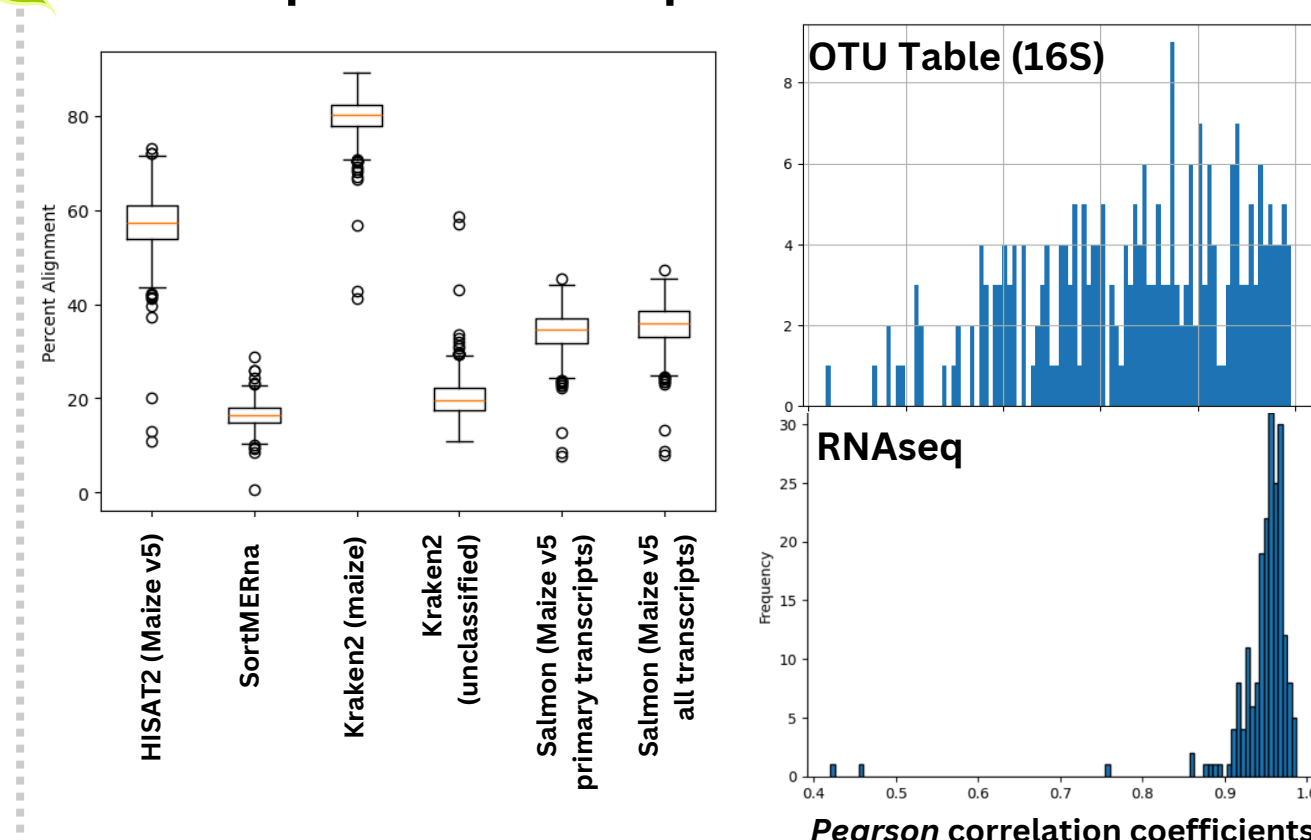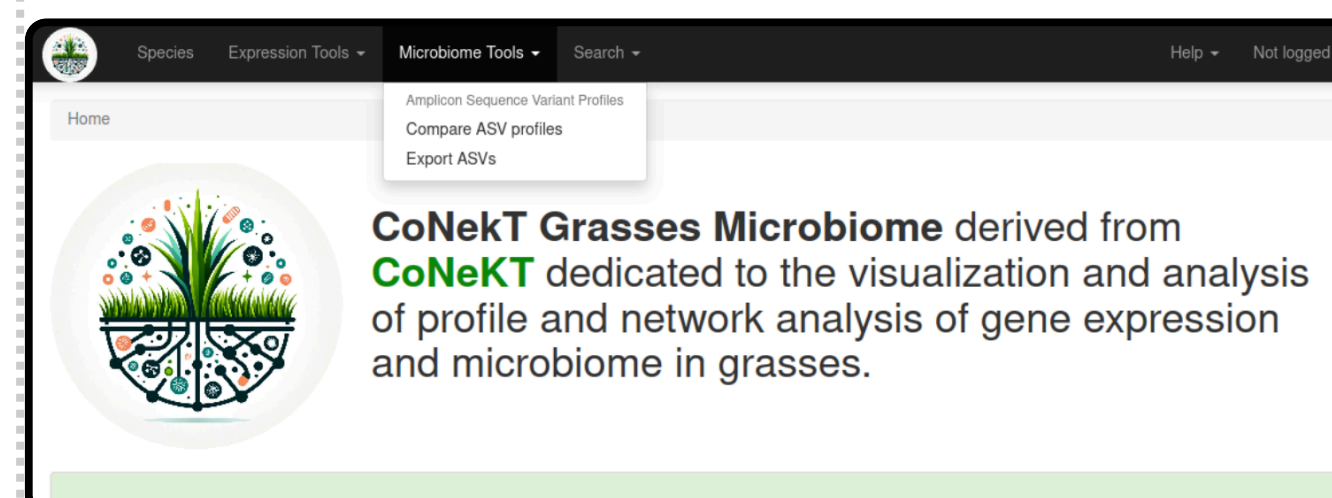
High read classification and mapping rates (maize B73 genome and transcriptome), and low rRNA contamination. Good correlation (*Pearson*) between night and day samples for the RNAseq TPM matrix and the OTU table (16S).

### Correlations microbiome - transcriptome



Correlated Pairs / Unique OTUs / Unique Transcripts
**Day**   **Night**

**Transcripts**
Night 187 | 63 | 210 Day
**OTUs**
35 | 42 | 20

### CoNekT Grasses Microbiome



**CoNekT Grasses Microbiome** derived from **CoNeKT** dedicated to the visualization and analysis of profile and network analysis of gene expression and microbiome in grasses.

Initial version of the web platform, which derives from CoNekT Grasses (http://conekt.cena.usp.br). Both were developed by adapting and enhancing pre-existing codebase of CoNekT (Proost & Mutwil, 2018).

**GitHub Wiki page**

## References

- Wallace, Jason G., et al. "Quantitative genetics of the maize leaf microbiome." Phytobiomes Journal 2.4 (2018): 208-224.
- Kremling, Karl AG, et al. "Dysregulation of expression correlates with rare-allele burden and fitness loss in maize." Nature 555.7697 (2018): 520-523.
- Proost, Sebastian, and Marek Mutwil. "CoNekT: an open-source framework for comparative genomic and transcriptomic network analyses." Nucleic acids research 46.W1 (2018): W133-W140.